Genetic and Molecular Ecotoxicology: A Research Framework

Susan Anderson,[1] Walter Sadinski,[1] Lee Shugart,[2] Peter Brussard,[3] Michael Depledge,[4] Tim Ford,[5] JoEllen Hose,[6] John Stegeman,[7] William Suk,[8] Isaac Wirgin,[9] and Gerald Wogan[10]

[1]Lawrence Berkeley Laboratory, Berkeley, California; [2]Oak Ridge National Laboratory, Oak Ridge, Tennessee; [3]University of Nevada Reno, Reno, Nevada; [4]University of Plymouth, Plymouth, UK; [5]Harvard University, Cambridge, Massachusetts; [6]Occidental College, Los Angeles, California; [7]Woods Hole Oceanographic Institute, Woods Hole, Massachusetts; [8]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [9]New York University Medical Center, Tuxedo, New York; [10]Massachusetts Institute of Technology, Cambridge, Massachusetts

Participants at the Napa Conference on Genetic and Molecular Ecotoxicology assessed the status of this field in light of heightened concerns about the genetic effects of exposure to hazardous substances and recent advancements in our capabilities to measure those effects. We present here a synthesis of the ideas discussed throughout the conference, including definitions of important concepts in the field and critical research needs and opportunities. While there were many opinions expressed on these topics, there was general agreement that there are substantive new opportunities to improve the impact of genetic and molecular ecotoxicology on prediction of sublethal effects of exposure to hazardous substances. Future studies should emphasize integration of genetic ecotoxicology, ecological genetics, and molecular biology and should be directed toward improving our understanding of the ecological implications of genotoxic responses. Ecological implications may be assessed at either the population or ecosystem level; however, a population-level focus may be most pragmatic. Recent technical advancements in measuring genetic and molecular responses to toxicant exposure will spur rapid progress. These new techniques have considerable promise for increasing our understanding of both mechanisms of toxicity on genes or gene products and the relevance of detrimental effects to individual fitness. — Environ Health Perspect 102(Suppl 12):3–8 (1994)

Key words: genetic, molecular, ecotoxicology, biomarkers, genotypes, genotoxic, global pollution, populations, ecosystems, biodiversity, hazardous substances

Introduction

The Napa Conference on Genetic and Molecular Ecotoxicology provided an opportunity to assess the field of genetic and molecular ecotoxicology in light of important recent developments. First, there is a growing sense of urgency regarding global pollution, which challenges us to assess subtle sublethal effects with the most informative diagnostic and prognostic techniques available. Second, interest in the preservation of biodiversity has heightened concerns that effects of hazardous substances on genetic variability are not well understood. Third, there has been rapid advancement of new molecular approaches for analysis of gene structure and function as well as improved validation of more classical techniques such as cytogenetic analyses. These developments set the stage for new research emphasizing broad goals and applications across levels of biological organization. Thus, these concepts must be integrated into a more comprehensive, contemporary definition of genetic ecotoxicology.

The goals of this article are to identify a more inclusive identity and a modern framework for future research in genetic and molecular ecotoxicology. We present the consensus of the group at the Napa Conference, but also highlight the diversity of opinion on key topics. We present the following: a proposed definition of the field, its potential relevance, key goals, core challenges, and a framework for future research.

Definition

We propose that genetic ecotoxicology may best be defined as:

"The study of chemical- or radiation-induced changes in the genetic material of natural biota. Changes may be direct alterations in genes and gene expression or selective effects of pollutants on gene frequencies."

Thus, this definition attempts to encompass direct DNA damage, epigenetic effects, and changes in gene pools attributable to toxicant exposure. In the past, genetic ecotoxicology has been identified primarily with the study of direct DNA damage. Semantic problems could stem from efforts to expand the definition of genotoxic response to embrace many sorts of molecular change. Therefore, we anticipate that the proposed definition will serve as a focus for future debate. Only recently have attempts been made to place the consequences of "pollutant-induced changes in genetic material" in a broader, more environmentally relevant context (*1*).

This article synthesizes findings of the Napa Conference on Genetic and Molecular Ecotoxicology held 12–15 October 1993 in Yountville, California.

This conference was supported primarily by the National Institute of Environmental Health Sciences (NIEHS) Superfund Basic Research Program (W. Suk, Program Director). Additional support was provided by the University of California, Berkeley, NIEHS Superfund Program Project (M. Smith, Principal Investigator, S. Neal, Project Administrator) and by an award from the Pew Scholars in Conservation and the Environment (S. Anderson, Principal Investigator). We are indebted to the many participants in the conference whose voices are expressed throughout this review.

Address correspondence to Dr. Susan Anderson, Bldg. 70-193A, Lawrence Berkeley Laboratory, 1 Cyclotron Road, Berkeley, CA 94708. Telephone (510) 486-4654. Fax (510) 486-5401.

Relevance

Hazardous substances are distributed widely in ecosystems due to diverse human activities such as energy usage, industrial enterprises, agriculture, and activities of the defense industry. The long-term ecological effects of these substances, as well as effects of increasing ultraviolet-B (UV-B) radiation as a consequence of ozone depletion, are largely unknown. For example, ecosystem protection is based largely on assessments of lethality in model organisms rather than on an understanding of toxic effects *in situ* on natural populations. Similarly, damage assessments of catastrophic events such as oil spills have, until recently (*2*), relied on mortality censuses of exposed animals and have not often considered sublethal genotoxicity. Although ozone depletion has received enormous international attention, only a few studies have examined the damaging effects of increased UV-B on DNA of wild organisms, even in the affected Antarctic ecosystem (*3*). In agricultural settings throughout the world, genotoxic compounds are used as pesticides and herbicides, yet we know that some of these substances can elicit adverse genetic and epigenetic responses in nontarget populations (*4*). Additionally, global mining practices have mobilized tons of hazardous mutagenic metals such as arsenic, mercury, nickel, and chromium into the environment where they are now potentially serious toxic threats. The ecological effects of these mining activities remain poorly understood.

In the human health arena, the consequences of exposure to genotoxic substances have been researched intensively for decades. In contrast, the level of scrutiny directed towards genetic ecotoxicology has been more fragmented. This situation is somewhat surprising given the potential for animals in ecosystems to serve as sentinels for effects in humans.

Nevertheless, documentation of genotoxic effects in ecosystems attributable to hazardous substances has emerged (*5*). This has recently included reports of tumors in aquatic species (*6*), truncated age classes in populations exhibiting increased incidence of tumors (*7*), damage assessments linking genotoxic effects of oil spills and subsequent mortality in commercial fish species (*2*), and findings implicating epigenetic effects as the cause of endocrine and reproductive disruption in a range of species (*4*).

Undoubtedly, however, other effects remain undetected. It is easier to detect tumors in an animal than to characterize long-term alterations of its reproductive potential. Yet, reproductive alterations are potentially some of the most important sublethal impacts of exposure to genotoxic substances (*8*). As the state of the art of research in ecotoxicology advances, we hopefully will be able to document more such effects and improve our capabilities to predict effects on populations.

Examples of changes in gene pools attributable to pollutant exposure are available in an underappreciated, diverse literature. Well-known examples, such as those of industrial melanism, pesticide tolerance, and tolerance to metals in numerous organisms have been reviewed (*9*). Recently attention has been directed toward the effects of pollutants on gene pools of fish (*10*) and natural microbial communities (*11*). New molecular capabilities should heighten the impact of such research.

We now recognize that pervasive global pollution requires that the effects of hazardous substances on biodiversity be well-studied. Several lines of evidence have shown that pollutants cause decreased genetic variability in populations and that such decreases are detrimental to population viability (*10,12*). Others (*13–16*) have raised the concern that pollutants could affect evolutionary processes. Thus, it is vital that we consider research in genetic ecotoxicology as crucial to conserving the genetic variability contained within ecosystems (*17*). How can we predict the loss of variability? How can we optimize restorations? How can we communicate that there is no going back once genetic variation has been eliminated?

It is not possible to assess genotoxic risks in detail in all species and populations. Thus, model species will continue to be highly important in establishing the mechanistic basis and validation of diagnostic probes, and disclosing phylogenetic differences in resistance or susceptibility. Applying such knowledge to analyses of sentinel species may then indicate potential hazards to other members of an ecosystem and even to humans (*18,19*). Studies of *ras* oncogenes in winter flounder (*20*), molluscan germinomas (*21*) and responses to Ah-receptor agonists in diverse vertebrates (*22*) all indicate a similarity of toxic mechanisms in many groups of animals and suggest that effects on sentinel species might indicate effects on other species as well. It is vital that we continue to determine the associations between responses in sentinel species and potential effects in humans.

Further research in genetic ecotoxicology could provide numerous benefits directed at improved management of hazardous substances in the environment. Biomarkers alone could be used to provide historical information on individuals and populations affected by hazardous exposure, identify species at risk due to exposure, derive cleanup targets for site remediation, assess effects of contaminants on the genetic diversity of populations, and measure effects in sentinel species as potential indicators of human exposure to chemicals present at Superfund sites.

Goals and Challenges

We propose that the overall goal of genetic ecotoxicology is best described as follows:

"To assess, predict, and prevent significant radiation- or chemical-induced genetic and epigenetic damage in populations."

Practically, attaining this goal will be demanding, in part because "significant genetic damage" is difficult to define and therefore difficult to measure, and because our ability to predict the consequences of such change is inadequate.

There are at least two possible definitions of "significant genetic damage." The first definition is based on a population-level focus:

Definition 1. Significant genetic damage is defined as altered genetic structure or function in individuals that ultimately results in decreased population abundance or irreversible changes in the genetic variability within gene pools.

This definition is attractive for several important reasons. The population is the level at which evolutionary processes are manifest; thus this definition provides a theoretical context for examining genetic alterations. The development of approaches to associate effects on individuals with effects on the population continues (*16*). Many of these are discussed below and in papers within this issue (*2,8*). Thus, it is tempting to embrace a population-level focus for describing significant genetic damage.

A broader, but more unwieldy, definition of "significant genetic damage" embraces an ecosystem-level focus:

Definition 2. Significant genetic damage is defined as altered genetic structure or function in individuals that ultimately results in disruption of an ecosystem and loss of ecosystem functional diversity.

This approach encompasses a more holistic view recognizing the connectivity among ecological processes and the scale at which ecological effects occur. However, many of the ecosystem-level interactions remain poorly defined and difficult to measure, making predictions beyond popula-

tions difficult at best. Thus, it is much more difficult to superimpose this somewhat vague concept of significant genetic damage onto an already amorphous paradigm. Nevertheless, critical environmental challenges, such as ecosystem restoration, dictate that genetic ecotoxicologists broaden their perspective to include thinking about the enormous complexity of ecosystems. This approach provides a stimulus to do that.

Differences in opinion regarding the appropriate definition of "significant genetic damage" will continue. In the meantime, the definitions provided here could serve as focal points for debate.

Another key barrier to the overarching goal of genetic ecotoxicology is that despite recent technological advances, the usefulness of biomarkers of genotoxicity for predicting effects on fitness and populations is in need of further validation. The following section addresses research needs for enhancing the use of biomarkers.

Research Framework

The evolving conceptual and technical foundation of genetic ecotoxicology calls for research that encompasses a broad view of the consequences of genotoxic exposure. Ultimately, we need to test hypotheses focused on mechanisms and effects across levels of biological organization, from that of DNA to populations and, ideally, even to ecosystems. Such a synthesis undoubtedly will require more interdisciplinary collaboration, including cooperative efforts between molecular biologists, ecotoxicologists, population geneticists, and community ecologists to forge comprehensive studies of genetic alterations in wild populations.

A conceptual model was formulated based upon the general consensus that more research should be focused on linkages between mechanisms and effects across levels of biological organization (Figure 1). Thus, the model begins with biomarker responses and ends with populations. It includes three types of genotoxic syndrome as primary examples of outcomes of genotoxic exposure: malignant tumors, decreased reproductive success, and altered genotypic diversity. We use these examples in the following discussion to illustrate the predictive potential of biomarkers and an interdisciplinary framework that emphasizes linkages in genotoxicological research.

Ideally, biomarkers that indicate molecular or genetic change would have predictive value related to organismal fitness. Biomarkers are measured in individuals and are thus less obviously related to effects that are measured on populations, but it is apparent that there are strong associations between changes in frequencies of biomarker responses in individuals and higher-order effects (*8*).

In any discussion of the predictive capacity of biomarkers, the potential importance of new molecular techniques must be emphasized. Researchers in genetic ecotoxicology have begun to capitalize on the rapidly advancing technology in molecular biology (*7,15,23*). As more critical genes are identified, and the functions of their products are understood, studies to elucidate mechanisms of effect can be greatly accelerated. In addition, there are opportunities to understand the significance of structural alterations in genes in terms of protein function in processes critical to survival or reproductive success.

Hopefully, an improved understanding of mechanisms of effect can be coupled to molecular characterizations of genotypic diversity at specific loci, with the intent of determining genotypic susceptibility to exposure and resultant effects on genotypic diversity in populations. We can then attempt to understand what altered DNA or reduced reproductive success in intolerant individuals means in terms of the resiliency, and hence persistence, of populations. Thus, advances in molecular technology have contributed greatly to a new synthesis in genetic ecotoxicology that integrates key response parameters along a continuum from DNA to populations. It is also likely that future gains in genetic ecotoxicology will depend increasingly on these techniques. This realization is critical when considering the linkages depicted in our conceptual model (Figure 1).

Potential relationships between biomarkers of genotoxicity and malignant tumors have been suggested by research conducted on fish and in the area of human health. In addition, parallels in the sequence from metabolic activation of procarcinogenic sites of adduct formation in DNA, and mutations in oncogenes and suppressor genes, have been established in mammalian and nonmammalian vertebrates (*18*). Thus, cancer represents an identifiable disease that can be linked to chemical exposure, and for which present mechanistic understanding has provided molecular markers.

However, sublethal effects on reproductive success might be far more pervasive, and not yet appreciated fully by genetic ecotoxicologists. Research has been conducted on linkages between biomarkers of early biolog-

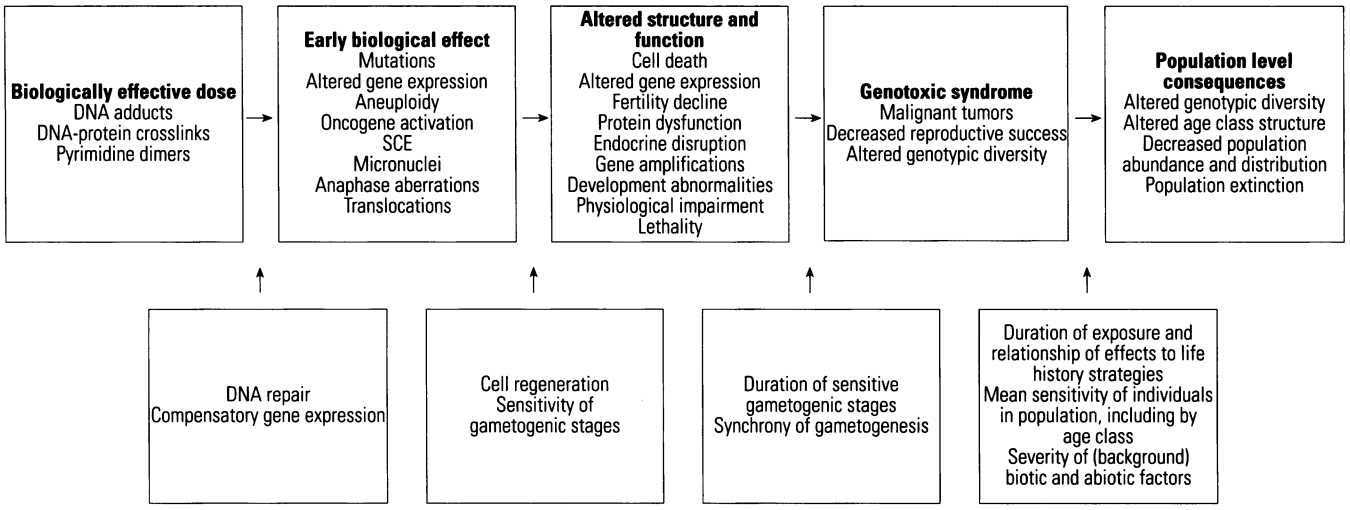

Figure 1. Possible ramifications of genotoxic exposure.

ical effect and either altered structure and function or decreased reproductive success (*8*). In contrast, little has been done to assess relationships between biomarkers of biologically effective dose and any measure of reproductive success. However, evidence that relates alterations in reproductive success to changes at the population level (*2*) indicate the need for such markers.

Numerous specific avenues of research could strengthen the use of biomarkers to predict reproductive effects. For example, promising future research might include measures of alterations in specific genes which are known to result in dysfunctional gametes or abnormal embryonic development. In general, there is a need for studies of linkages between exposure to contaminants, increases in frequencies of biomarkers, and reduced reproductive success with a select array of contaminants, biomarkers, and species. One means of accomplishing this goal is the increased application of biomarker techniques directly in germ cells and embryos. More comprehensive documentation of such relationships is vital in order to address questions of whether biomarkers of sublethal DNA damage are indicative of effects on fitness.

There are obstacles to addressing these needs, due primarily to the difficulty of measuring biomarkers and reproductive success in exposed feral populations, but we posit that more could be done with those populations and species that are experimentally tractable (*24*) and more should be attempted with those that are not. There is an urgent need to assess genotoxic impacts across taxa in wild populations under environmentally realistic conditions (*25,26*). Nondestructive biomarkers are vitally important in this regard, especially with respect to sampling of threatened or endangered populations (*27*).

Alterations in genotypic diversity are another potential effect of exposure to genotoxicants. Not many genetic ecotoxicologists have studied effects of toxicant exposure on gene pools (*10,16*). Unfortunately, not many population geneticists, in whose realm studies of genotypic frequencies usually fall, have considered the effects of toxic pollution on genetic variability either. In addition, the role and magnitude of interindividual variability is poorly understood (*16,28*). It is time to recognize that, at a minimum, genetic ecotoxicologists are interested in conserving populations of currently or potentially exposed species and therefore must think beyond effects on individuals. Likewise, population geneticists must incorporate a broader spectrum of mechanisms by which genetic variability can be reduced, including toxicant exposure. Thus, in terms of understanding the most critical effects of genotoxic exposure in nature, there is considerable need for genetic ecotoxicologists and population geneticists to join efforts.

The principles underlying research on effects of genotoxicants on genotypic diversity are not new (Figure 2). In a highly heterozygous population, there are likely to be certain genotypes that are more sensitive to genotoxic exposure than others. This is especially true if this population is heterozygous at loci that are both critical to fitness and susceptible to toxicant-induced structural alterations. Genotoxic exposure can act as a selective force by eliminating sensitive genotypes, or by reducing the number of offspring that they contribute to the next generation. The result is a reduction in the total genetic variation within that population or a shift in genotypic frequencies. It is generally accepted that genetic variation provides the requisite flexibility for a population to persist in the face of variable biotic and abiotic selective forces over time. Reduced variation can thus lead to increased rate of extinction.

Research into toxic effects on genotypic diversity can be conducted in a variety of ways. One of the potentially most productive approaches would be to: *a*) use biomarkers to identify individuals sensitive to exposure (*29*); *b*) determine the genotypes of these individuals at critical loci; *c*) determine the genotypes of tolerant individuals at these same loci; *d*) determine whether certain alleles at these loci conferred greater fitness and, ideally, why that was the case; and then *e*) determine the frequencies of genotypes at these loci in exposed and unexposed populations. This might be particularly easy to address at the microbial level.

Similar approaches have been pursued by examining suites of allozymes in exposed and unexposed populations (*10*). The major improvement that the suggested approach offers over past efforts is one of specificity. Due to technological advances at the molecular level, it is conceivable to be able to identify specific critical genes or gene products such as the *p53* gene or cytochrome P450 enzymes (*30,31*), to relate genotypes at these loci to fitness, and subsequently to relate fitness to overall heterozygosity at these loci in the population.

To facilitate documentation of whether or not these more specific techniques and relationships can be important and useful, perhaps collaborative, multilevel studies with classic model systems should be undertaken. These studies could serve as prototypes for research with less well-studied organisms and broaden the technical scope of theoreticians and empiricists alike. Eventually, models could be formulated that predict effects of genotoxicants on wild populations.

There are a number of other issues that must be considered regardless of the particular effect of genotoxic exposure under scrutiny. These include: *a*) concentrations of some toxicants in the environment will likely increase on a global scale; *b*) toxicants occur as complex mixtures; and *c*) chemical compounds are often present at low, yet possibly toxic concentrations. Most studies thus far have examined effects from the perspective of high-dose, acute exposures to single toxicants. We know very little about effects of chronic exposure to lower levels of multiple mutagens that are pervasive and persistent in the environment. As global pollution problems gain more attention, we will also be forced to determine the significance of low-level responses in comparison to background levels of factors that might elicit these responses. This represents an exact parallel to the important debate over food mutagens in human health risk assessment. In ecotoxicology, little has been done to address this problem (*32,33*).

The heritability of toxicant-induced mutations and the importance of mutational load have not been well-studied. Remarkably, theoretical treatments of fixed mutations and mutational load have not

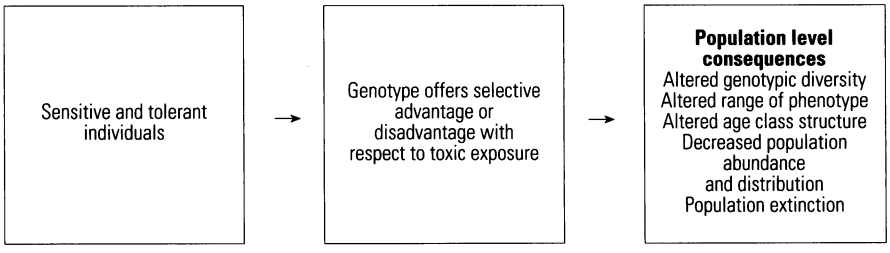

Figure 2. Potential genotoxic effects on genotypic diversity.

considered the potential effects of toxicant exposure. For example, contaminants may alter the timescales in which mutational load accumulates and thus alter the relative significances of natural selection and drift as evolutionary forces. This is especially true regarding asexually reproducing and hermaphroditic species, as well as very small populations of animals with sexual reproduction. In addition, contaminants may affect the demographic features of populations, and these changes are not necessarily considered in existing theory.

Multigeneration effects of toxicant exposure have only recently come to the forefront of scientific and public thinking, due largely to concerns over pesticides that act as estrogen analogues (*4*). These compounds can induce epigenetic effects that are expressed in progeny of exposed parents. The effects are not only a function of dose, but also the developmental stage during which exposure occurred. Both multigeneration genetic and epigenetic effects must be considered more thoroughly if we are to comprehend what is surely a broad range of genotoxic effects.

Some of the more obvious factors that could modify genotoxic effects are presented in the conceptual model (Figure 1). Perhaps one of the more important ones to consider is the differential sensitivities of developmental stages in gametes and embryos, as well as differential sensitivities among species with different life history strategies. It is important that we understand whether effects can be underestimated if studies are conducted with organisms that are at a less sensitive age or with species from an exposed community that are not representative of effects within that community. Ideally, we would want to know the mechanisms dictating developmental or life-history sensitivity. These sorts of determinations could also help establish a more genuine, and general, usefulness of indicator or sentinel species.

In addition to the practical concerns of assessing genotoxicity in the present and predicting it in the future, attempts to reverse toxicity from the past will present challenging opportunities for genetic ecotoxicologists, as restorations of toxic sites are a prominent goal. Restorations illustrate the importance of establishing firm linkages between effects on individuals and effects on populations and of determining how specific tools can be employed to answer critical questions. For example, will we be able to predict which individuals will have maximum fitness in restored populations? It is within such applied contexts that the predictive value of biomarkers, molecular characterizations of genetic sensitivity, and hypothesized susceptibilities of relatively invariable gene pools to extinction, could get their severest tests. Eventually, we must understand better how toxicant-induced changes in DNA affect populations and communities (*17*).

Conclusions

Techniques in genetic ecotoxicology are in a rapidly evolving state such that reliable tools are now available for addressing more complex environmental problems. The increasing availability of reliable diagnostic tools will greatly improve our ability to assess the sublethal effects of exposure to hazardous substances. We must envision their promise for addressing these problems and identify the most urgent directions for future research. However, a broader view of the ecological consequences of exposure to hazardous substances, one that encompasses a multidisciplinary focus, is also needed. These concepts will be important in at least five critical areas of research, including restoration ecotoxicology, effects of global environmental change, effects of contaminants on the genetic diversity of populations, multilevel assessments of the predictive capability of biomarkers, and linkages between molecular changes in animal sentinels of environmental toxicity and implications for human health. To succeed in establishing a more multidisciplinary focus, we must improve the predictive capability of biomarker techniques with respect to assessing effects on populations. An understanding of molecular mechanisms will provide a foundation for the use of biomarkers in this regard, but we also need to understand mechanisms of effect at all levels of biological organization. Ultimately, understanding of the linkages between molecular and biochemical responses to toxic exposure and effects on individual fitness, populations, and ecosystems must be integrated with theories from population genetics and ecology to form a broader model for assessing and predicting effects of environmental pollution.

REFERENCES

1. Wurgler FE, Kramer PGN. Environmental effects of genotoxins (eco-genotoxicology). Mutagenesis 7:321–327 (1992).
2. Hose JE. Large-scale genotoxicity assessments in the marine environment. Environ Health Perspect 102(Suppl 12):29–32 (1994).
3. Karentz D. Considerations for evaluating ultraviolet radiation-induced genetic damage relative to Antarctic ozone depletion. Environ Health Perspect 102(Suppl 12):61–64.
4. Colborn T. The wildlife/human connection: modernizing risk decisions. Environ Health Perspect 102(Suppl 12):53–57 (1994).
5. Stegeman JJ, Brouwer M, DiGiulio RT, Forlin L, Fowler BA, Sanders BM, Van Veld PA. Molecular responses to environmental contamination: enzyme and protein systems as indicators of contaminant exposure and effect. In: Biomarkers for Chemical Contaminants (Huggett RJ et al., eds). Boca Raton, FL:Lewis Press, 1992;235–335.
6. Moore MJ, Myers MS. Pathobiology of chemical-associated neoplasia in fish. In: Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives (Malins DC, Ostrander GK, eds). Boca Raton, FL:CRC Press, 1994;327–386.
7. Wirgin II, Garte SJ. Assessment of environmental degradation by molecular analysis of a sentinel species: Atlantic tomcod. In: Molecular Environmental Biology (Garte SJ, ed). Boca Raton, FL:Lewis Publishers, 1994;117–132.
8. Anderson SL, Wild GC. Linking genotoxic responses and reproductive success in ecotoxicology. Environ Health Perspect 102(Suppl 12):9–12 (1994).
9. Bishop JA, Cook LM. Genetic Consequences of Manmade Change. London:Academic Press, 1981.
10. Guttman SI. Population genetic structure and ecotoxicology. Environ Health Perspect 102(Suppl 12):97–100 (1994).
11. Ford T. Pollutant effects on the microbial ecosystem. Environ Health Perspect 102(Suppl 12):45–48 (1994).
12. Nevo E, Noy R, Lavie B, Beiles A, Muchtar S. Genetic diversity and resistance to marine pollution. Biol J Linn Soc 29:139–144 (1986).
13. Dieter MP. Identification and quantification of pollutants that have the potential to affect evolutionary processes. Environ Health Perspect 101(Suppl 4):278 (1993).
14. Thaler DS. The evolution of genetic intelligence. Science 264:224–225 (1994).
15. Bickham JW, Smolen MJ. Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. Environ Health Perspect 102(Suppl 12):25–28 (1994).
16. Depledge M. Genotypic toxicity: implications for individuals

and populations. Environ Health Perspect 102(Suppl 12): 101–104 (1994).
17. Stomp A-M. Genetic information and ecosystem health: arguments for the application of chaos theory to identify boundary conditions for ecosystem management. Environ Health Perspect 102(Suppl 12):71–74 (1994).
18. Dawe C, Stegeman JJ. Symposium on chemical contamination of aquatic food resources and human cancer risk. Environ Health Perspect 90:1–154 (1991).
19. Wirgin II, Grunwald C, Courtenay S, Kreamer G, Reichert WL, Stein JE. A biomarker approach to assessing xenobiotic exposure in Atlantic tomcod from the North American Atlantic coast. Environ Health Perspect 102(Suppl 9):2–8 (1994).
20. McMahon G, Huber LJ, Moore MJ, Stegeman JJ, Wogan GN. Mutations in C-K-*ras* oncogenes in diseased livers of winter flounder from Boston Harbor. Proc Natl Acad Sci USA 87:841–845 (1990).
21. Van Beneden RJ. Molecular analysis of bivalve tumors: models for environmental/genetic interactions. Environ Health Perspect 102(Suppl 12):71–74 (1994).
22. Hahn ME, Poland A, Glover E, Stegeman JJ. Photoaffinity labeling of the Ah receptor: phylogenetic survey of diverse vertebrate and invertebrate species. Arch Biochem Biophys 310:218–228 (1994).
23. Roesijadi G. Metallothionein induction as a measure of response to metal exposure in aquatic animals. Environ Health Perspect 102(Suppl 12):91–96 (1994).
24. Shima A, Shimada A. The Japanese medaka, *Oryzias latipes*, as a new model organism for studying environmental germ-cell mutagenesis. Environ Health Perspect 102(Suppl 12):33–35 (1994).
25. Stein JE, Reichert WL, Varanasi U. Molecular epizootiology: assessment of exposure to genotoxic compounds in teleosts. Environ Health Perspect 102(Suppl 12):65–69 (1994).
26. Dickerson RL, Hooper MJ, Gard NW, Cobb GP, Kendall RJ. Toxicologic foundations of ecologic risk assessment: biomarker development and interpretation based on laboratory and wildlife species. Environ Health Perspect 102(Suppl 12):65–69 (1994).
27. Fossi MC. The use of nondestructive biomarkers in ecotoxicology. Environ Health Perspect 102(Suppl 12):49–54 (1994).
28. Courtenay S, Williams PJ, Grunwald C, Konkle B, Ong T-L, Wirgin II. Assessment of within-group variation in CYP1A mRNA inducibility in environmentally exposed and chemically treated Atlantic tomcod. Environ Health Perspect 102(Suppl 12):85–90 (1994).
29. Shugart L, Theodorakis C. Environmental genotoxicity: probing the underlying mechanisms. Environ Health Perspect 102(Suppl 12):13–17 (1994).
30. Stegeman JJ, Lech JJ. Monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen exposure. Environ Health Perspect 90:101–109 (1991).
31. McMahon G. The genetics of human cancer: implications for ecotoxicology. Environ Health Perspect 102(Suppl 12):75–80 (1994).
32. Vrolijk NH, Targett NM, Woodin BR, Stegeman JJ. Toxicological and ecological implications of biotransformed enzymes in the tropical teleost, *Chaetodon capistratus*. Mar Biol 119:151–158 (1994).
33. Anderson SL, Hoffman JR, Wild GC, Bosch I, Karentz D. Cytogenetic, cellular and developmental responses in Antarctic sea urchins following laboratory ultraviolet-B and ambient solar radiation exposures. Antarc J (in press).